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Plant Ecotoxicology: The Design and Evaluation of Plant Performance in Risk Assessments and Forensic Ecology

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ABSTRACT: Since the emergence of ecology as a serious discipline, debates on the relevance of laboratory studies including toxicology have been prominent. Standardized phytotoxicity tests are often challenged due to the limited range of test species and the narrow range of environmental conditions proscribed in the tests. This paper discusses the uncertainty currently associated with lab-to-field extrapolations and other aspects of standardized tests. Characterization of test parameters is critical as a first step to quantification and, ultimately, reduction of uncertainty. A conceptual context for designing studies to quantify and reduce uncertainty is presented. A step-wise procedure based on Koch's postulates is offered as the best way to bolster linkage of toxicity data to predictions of risk and characterization of posts incidents (forensic ecology).

KEYWORDS: phytotoxicity, risk assessments, forensic ecology, uncertainty

Characterizing hazard of contaminated environmental samples, toxic chemicals, and pesticides has led to the development of a select set of standardized test procedures for plants [16]. The linkage between innate hazard (laboratory determination of toxicity) and ecological risk often poses unacceptable levels of uncertainty. The tools, to acquire the primary data that are used in Ecological Risk Assessments (EcoRAs; predictive), and forensic ecology (investigation of past events and incidents) come from classical methods in ecological sampling (biosurveys, field monitoring, biogeography) and standardized laboratory toxicity tests [10]. The integration of toxicity data and exposure

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estimates is constrained by the power (or lack thereof) of the primary data. The principal deficiencies of standardized tests *vis-a-vis* ecological risk assessments relate to exposure conditions, relevant endpoints, interspecies extrapolation, and limited lab-to-field extrapolation. Fundamental changes that broaden the types and scope of "standardized" tests and expand the suite of measurement endpoints are needed. This next generation of standardized tests must be developed in light of ecological risk assessment requirements and expectations, if they are to be effective.

In considering the suite of standardized phytotoxicity tests available, it is important to recall that the tests were developed before the current ecological risk assessment procedures had evolved. The primary uses of the "First Generation" phytotoxicity tests (Table 1) provide limited information in comparison to the issues addressed in EcoRAs.

Table 1 -- Purpose, expectations, and requirements of toxicity tests and EcoRAs.

"First Generation" Toxicity Tests	**Ecological Risk Assessment Issues**
• screen commercial chemical(s) for potential adverse effects on organisms • integrate the effects of multiple chemicals (mixtures) on organisms • evaluate the effect of chemical(s) in environmental media • monitor effects of point-source discharges • compare relative toxicity of different chemicals • compare species sensitivity to given chemicals	• anticipate bio-availability • characterize relevant exposure parameters • relate avoidance-acclimation-adaptation continuum to effects • consider the relative importance of lethal vs. non-lethal effects • extrapolate quantitative effects on individuals to population and higher levels of ecological organization • segregate multiple stressor effects • identify and characterize cascading (indirect) effects • consider and anticipate long-term effects

Nevertheless, EcoRAs rely extensively on toxicity data from standardized tests. In simplified form, the EcoRA considers the innate toxicity of individual chemicals on ecological resources, evaluates the likelihood of exposure applicable to specified pathways in site-specific situations or proscribed scenarios, and combines the toxicity and exposure information to quantify the risk to ecological resources [17]. Under recent guidelines, the EcoRA focus has been expanded to consider non-chemical stressors (e.g., physical disturbance, temperature, drought, or herbivory).

Incorporating standard toxicity information into EcoRAs has generated much anxiety regarding the ecological relevance and relative significance of other stressors. A comparison of the design criteria

for toxicity tests and the expected parameters for EcoRAs (Table 2) offers some justification for the anxiety and frustrations. In an ideal situation, toxicity and exposure data would be available for sensitive taxa and the endpoints of concern. Typically, neither toxicity nor exposure data are available for the taxa or the endpoints of concern.

Table 2 -- Comparison of toxicity test design criteria and EcoRA parameters.

Design Consideration of "First Generation" Phytotoxicity Tests	Parameters for Ecological Risk Assessments
• laboratory based • test organisms readily available nationally (globally) throughout year • test organisms capable of nominal performance in lab • testing facilities, equipment, and supplies simplified to minimize cost • simple measurement endpoints • test procedures free of or minimally dependent on professional judgment • test results amenable to standardized statistical treatment • report results simplified and routine • short exposure times (days)	• field oriented • endemic or naturalized populations and communities in site-specific or regional settings • ecological setting exhibits high-levels of complexity with interdependence of multiple, typically interacting abiotic and biotic factors that may not have known nominal performance standards • complex, interactive ecological trajectory • ecological rules not yet formalized; requires substantial professional judgment • normal statistics likely to be inappropriate • EcoRA must be reported in terms relevant to risk management

The phytotoxicity literature contains a mix of standard laboratory tests, laboratory/greenhouse experiments, field experiments, and field surveys (gathering "baseline" soil concentration-plant effects information; see reviews by, 6,7,10). Since the objectives of these studies are varied, seldom is the information in a consistent format that allows straightforward integration of information into an ecological risk assessment. Critical information may not be reported (either in the primary literature or the secondary literature); alternatively the information may be too detailed to readily allow comparison among different source information (See [14, 3]). The interpretation of information, either by expert professionals or through computer-aided analysis, requires the development of categories to group data regardless of the level of detail provided. The task can be daunting.

In recent years, there has been progress made in defining general relationships and uncertainty inherent in extrapolation from general literature, including the phytotoxicity literature, to risk assessments [3, 4, 10]. Many critical factors, however, remain unresolved (Table 3). In that many highly qualified individuals and groups have attempted to extract relationships from the vast literature, it is reasonable to

conclude that data may not contain the information needed to reduce uncertainty in EcoRAs to any great extent.

At the surface of the problem, there are apparent philosophical conflicts. Whereas advances in toxicology rely on reductionism (e.g., pharmicokinetics and structure activity relationships), the push in risk assessment is toward holistic approaches. Ecotoxicology must navigate both arenas. Attempting to bridge these gaps, research has offered several potential approaches; many conflict with one or more alternatives. In addition, it is important to reflect on underlying philosophical and pedagogical considerations.

Ecologists have debated issues of scale in one form or another since the inception of the discipline. Efforts to develop manageable studies have oscillated between competing forces to simplify ecosystem issues on the one hand and incorporate sufficient complexity to controlled tests on the other hand. From this emerged the terminology and protocols for full-scale ecosystem or real world (field) studies, mesocosms, and microcosms. Each level of complexity and spatial scale have merit for certain problems (Table 4).

An alternative to more complex testing schemes is to tailor simple tests that are designed to address a specific data requirement for an EcoRA. Major technical achievements in science are, in retrospect, characteristically simple. Simplicity is achieved through development of focused questions. More emphasis needs to be placed on developing relevant questions that current techniques can answer. The initial standardized tests were developed in this manner to achieve specific answers. By and large, these tests have been effective for the series of questions asked in the pre-EcoRA days of regulation. With the advent of EcoRA, different questions are posed, ones that the initial standardized tests were not designed to answer. Special effort must be focused on dissecting the critical questions of EcoRA, and designing simplified, experimentally-based test modifications that build on the wealth of toxicity data generated over the past three decades. To be effective, this effort should consider advances in analytical tools, ecological principles, and adjustments in expectations in EcoRA.

Table 3 -- Data qualifiers and uncertainty factors considered in plant uptake and phytotoxicity records for use in risk assessments and forensic ecology..

Parameter	**Sub-Parameter**	**Qualifier**	**Uncertainty Factor**	**Reference/Rationale**
test condition	field - natural conditions	none	1	direct measure
	field - amendments	chemical only	?	direct measure, incorporation unknown
	field - amendments	with nutrients (fertilizers), lime, or sludge	?	pH and organic matter strongly influence bioavailability
	greenhouse/lab	based on pesticide studies	2 - 4x	2X captures 65% 4X captures 100% [4,10]
taxa -- organics, and non-essential elements [e.g., As, Cd, Cr, Se, Pb]				[1,4,10]
	same species	N/A	1	
	within genus	$r^2 = 0.868$	5	captures 59% of all variation
	within family	$r^2 = 0.559$	10	captures 77% of all variation
	within order	$r^2 = 0.134$	50	captures 98% of all variation
	within class	$r^2 = 0.081$	100	captures 98% of all variation
taxa -- essential elements [e.g., Cu, Fe, Mn, Zn]				[1,4,10]
	within genus	same as above	1	
	within family	same as above	3	guestimate lower because
	within order	same as above	5	metals are essential
	within class	same as above	10	elements and
	within Class	same as above	20	regulated by plants

Table 3 -- Continued

Parameter	Sub-Parameter	Qualifier	Weighting Factor	Reference/Rationale
phytotoxicity endpoints	death	1	?	• less important for uptake since this limits pathway to herbivores
	reduced growth (vegetative)	0.5	?	• may be more important to herbivores loading, affects plant interspecies dynamics
	reduced yield (reproductive)	0.5	?	• limits food source, may limit seedling recruitment, affects plant interspecies dynamics
	morbidity	0.25	?	• impairs growth & uptake, affects plant interspecies dynamics
	impaired physiology	0.25	?	• limits fitness, reduces resilience, affects plant interspecies dynamics
study length [should match to desired endpoints]	multi-generation	may represent genetic selection	?	• limited value for toxicity, may be important for uptake
	life cycle	addresses all phases of development	?	• important for toxicity and uptake values
	chronic < life-cycle	best for growth endpoints	?	• subject to acclimation; dilution by growth; integrates whole plant ecophysiology
	acute	provides "instantaneous" /short-term uptake & toxicity	?	• fewer unrelated parameters

Table 4 -- Complex Testing Approaches & Limitations

Full-Scale Ecosystem, Real World (Field) Studies

- Field observations are crucial, and under the rules of science, the ultimate determinator of inferences.
- Complexity of natural systems reflects the consequence of usually undefined historical events and the interplay of homeostasis, genetic selection, chance, ...
- Experimental inference requires the identification of matched reference sites in sufficient numbers to achieve statistical power dictated by study objectives.
- Experimental manipulation of large study sites effectively results in the irretrievable loss of potential field sites as each test would require a new set of sites.

Mesocosms

- Achieve most objectives of the full-scale studies, but suffer the same limitation of sites being dedicated to one study for truly effective analysis.

Microcosms

- Serious difficulty in achieving and verifying simulation of complexity assumed in the test design.

Other scientific problems have presented similar intellectual obstacles. A few decades ago, there were solid educational programs in the field of plant and animal physiological ecology. One of the important topics of the 1960s and 1970 was allelopathy; the chemical toxicity one plant exerts on another [13]. Practitioners of allelopathy adapted Koch's Postulates [5], the step-wise methodology first developed to characterize infectious disease organisms, to establish the presence of a toxic chemical, dose-response relationships, physiological mode of action, environmental concentration, and then verify effects in the field through weight-of-evidence processes. A similar approach is recommended for risk assessment and forensic ecology (Table 5). The construct of ecological risk assessment problems is virtually identical to those faced by physiological ecologists examining allelopathy. The major differences facing ecotoxicology today can be found in the technological, analytical, and conceptual developments of the past two decades. The underlying theme of this construct is the reliance on established scientific methodology. Conclusions are not based on paradigms of what ought to be; rather, they are based on testable (i.e., refutable) hypotheses.

Ecology has had a vibrant history since the early descriptions of succession in the 1890s marked the beginning of the discipline [12,15]. Ecology is completing its first century as a formal biological discipline. Several schools of thought have dominated this brief

history, often generating intense intellectual combat. Some of the more prominent themes have been Clements' Monoclimatic Climax, Daubenmire's Polyclimatic Climax, Gleason's Individualism that gave rise to the Continuum Concept of Cottom & Curtis. More recently, Systems Ecology and Landscape Ecology have been at the forefront.

It is humbling to consider that: <u>none has produced a unifying principle of ecology; none has provided fully satisfactory predictive power!</u> Consequently, as a discipline, we struggle with many critical unresolved issues. A few examples illustrate the void:

- Boundaries of assumed linear processes are generally unknown;
- Interactive feedback, resiliency, and transition boundaries are poorly understood;
- New analyses continue to challenge once widely accepted concepts -- diversity, keystone species, indicator species, recovery, succession, ...

In recent years, with the prominence of the environmental movement into daily politics, ecology has drifted from its strong scientific foundation, taking on a religious fervor that might be termed *Eco-theocracy*. If we are to succeed in developing improved EcoRAs, we must pay particular attention to the central constructs of ecology and its dogma. Given the limitations of ecological principles, grand-scale environmental programs are often erected on faith, rather than on scientific foundation. These deficiencies of basic ecological principles pose substantial limitations to EcoRA. To a significant extent, the EPA Framework for EcoRA has tacitly recognized some of these constraints [17]. Much remains to be done.

Previously, we argued the importance of accounting for uncertainty, and illustrated that risk assessment and forensic ecology requires verification at each level of extrapolation [8,9]. Absent verification of prediction or causality, "accuracy" can rapidly grow to eight orders of magnitude [unpublished risk assessment on dioxin by Williams and Kapustka]. We have also argued that environmental issues are determined on the basis of social and political convictions, with science having a subordinate if not insignificant role [2]. Nevertheless, risk assessments must strive to remain focused on scientific processes based on observation, experimentation, and analysis. To do otherwise risks our ability as a society to distinguish factual information from hyperbole. One of the greater myths embedded in policy decisions today is that it is best to adopt the most conservative regulation available. The argument goes that it is best to err on the safe side. This would be correct if there were not a societal cost involved in adopting that stance. Unfortunately, in the US and to some extent elsewhere, millions of dollars are being spent with no indication that the effort will improve the environment. Meanwhile, less glamorous projects are ignored that could make substantial improvements in human and non-human resource quality. Energy and money wasted on frivolous ventures are not available for important concerns.

Regardless of the choice between complex versus simplified tests, theoretical advances and improved computer technology now affords use of increasingly sophisticated analytical tools. Non-parametric cluster analysis, Monte-Carlo simulations that capture and retain statistical

descriptions of distributions, precision, accuracy, and uncertainty, and multi-dimensional attribute analysis are supplanting conventional statistical approaches. The advanced technologies permit the derivation of conclusions from primary data. All too often, the conventional analyses have been misused to fit conclusions to popular ecological paradigms rather than let the data "speak for itself."

To maximize the use of phytotoxicity data in EcoRAs, more attention should be devoted to the difficult tasks of defining assessment endpoints, establishing suitable measurement endpoints, and stipulating the data quality objectives -- (i.e., employing the scientific method), [9]. All this should be completed before undertaking any specific measurement or test procedure. Once these steps are completed, informed decisions can be made as to whether existing standard test procedures are appropriate or adequate. Given the disparity between the initial design features of standardized tests and the expectations for EcoRAs, we might anticipate the need for focused test methods that embody enough flexibility to characterize and quantify specific risk assessment parameters such as site-specific bioavailability, exposure, non-lethal effects, or inter-species comparability. Because few ecological "principles" have broad predictive power, we must strive to limit our enthusiasm for grand-scale predictions of ecological risk. We can, however, expect to make substantive incremental improvements in risk assessment capability if we return our focus to the scientific process.

Table 5 -- Step-wise methodology to establish chemical toxicity adapted from Koch's Postulates.

Koch's Postulates (disease identification)	**Modified Koch's Postulates** [chemical interference) (toxicity & forensic ecology)	**Modified Koch's Postulates** [chemical interference) (toxicity & predictive risk)
STEPS		
1. characterize symptoms of disease 2. isolate putative disease organism 3. characterize the putative disease organism in culture 4. inoculate (expose) healthy test organisms 5. observe symptoms in inoculated organisms	1. observe field conditions (injury); characterize the symptoms, magnitude, and extent of problem in the field 2. identify putative contaminants 3. characterize mode-of-action & symptomology 4. establish "dose-response" relationships 5. demonstrate the presence of putative toxic substances in the field within the "effects range" concentrations 6. demonstrate the opportunity for exposure	1. establish "dose-response" relationships 2. characterize mode-of-action & symptomology 3. characterize exposure parameters 4. estimate future environmental concentrations (taking into account fate & transport expectations) 5. postulate specific, measurable ecological effects 6. conduct testable field experiments (i.e., ones that link steps 5 and 6 of forensic investigations)
CONFIRMATION REQUIREMENTS		
• the inoculated organism must develop the disease symptoms • must re-isolate, re-culture, and confirm the identity of disease organism	• apply weight-of-evidence criteria to establish relationship between contaminant and observed effects • document the uncertainty of the conclusions (quantitative where possible, qualitatively as default position)	• quantify the probability of reaching specified ranges of environmental concentrations, the probability of associated ecological effects • document the uncertainty of both environmental concentration and effects predictions

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